



SEQUENCE LISTING

RECEIVED  
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TECH CENTER 1600/2900

<110> RIKEN

<120> A MUTANT KANAMYCIN NUCLEOTIDYLTRANSFERASE AND A METHOD OF  
SCREENING THERMOPHILIC BACTERIA USING THE SAME

*Sub*  
*B1*  
<130> PH-1082

<140>

<141>

<150> JP 11-309616

<151> 29-OCT-1999

*A*  
<160> 11

<170> PatentIn Ver. 2.0

<210> 1

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant enzyme obtained by introduction of point mutation into wild type KNT gene of *Staphylococcus aureus* and its expression

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Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val  
1 5 10 15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys  
20 25 30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr  
35 40 45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe  
50 55 60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Tyr  
65 70 75 80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp  
85 90 95

Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser  
100 105 110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala  
115 120 125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe  
130 135 140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr  
145 150 155 160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu

165

170

175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu

180

185

190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu

195

200

205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu

210

215

220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg

225

230

235

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His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe

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<210> 2

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant enzyme obtained by introduction of point mutation into wild type KNT gene of *Staphylococcus aureus* and its expression

<400> 2

Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

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His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys

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30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr

35

40

45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Gly Ala Glu Phe

50

55

60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr

65

70

75

80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Ser Asp Trp

85

90

95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro

100

105

110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala

115

120

125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe

130

135

140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr

145

150

155

160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu

165

170

175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu

180

185

190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Ser Gly Tyr Asp His Leu

195

200

205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu

210

215

220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg

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His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe

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<210> 3

<211> 253

<212> PRT

<213> Artificial Sequence

<220>

<223> mutant enzyme obtained by introduction of point mutation into wild type KNT gene of *Staphylococcus aureus* and its expression

<400> 3

Met Lys Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

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His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys

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30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr

35

40

45

Ser Asp Ile Glu Met Met Cys Val Leu Ser Thr Glu Gly Val Glu Phe

50

55

60

Ser Tyr Glu Trp Thr Thr Gly Glu Trp Lys Ala Glu Val Asn Phe Tyr

65

70

75

80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Arg Val Glu Pro Asp Trp

85

90

95

Pro Leu Thr His Gly Arg Phe Phe Ser Ile Leu Pro Ile Tyr Asp Pro

100

105

110

Gly Gly Tyr Phe Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala

115

120

125

Gln Lys Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe

130

135

140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr

145

150

155

160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu

165

170

175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu

180

185

190

Thr Glu Ala Val Lys Gln Pro Asp Leu Pro Pro Gly Tyr Val Gln Leu

195

200

205

Cys Gln Leu Val Met Ser Gly Gln Leu Ser Asp Pro Glu Lys Leu Leu

210

215

220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Val Gln Glu Trp Ala Glu Arg

225

230

235

240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe

245

250

<210> 4

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 5' -Primer for PCR  
amplification

<400> 4

gactgtacgg gtacccgttg acggcggata tggtta

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<210> 5

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 3'-Primer for PCR  
amplification

<400> 5

gactgtacgc tgcagcgtaa ccaacatgtat taaca

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<210> 6

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: 5'-Primer for PCR  
amplification

<400> 6

gactgtacgg aattcgagct cgagcaaatc taaaa

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<210> 7

<211> 35

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:5'-Primer for subcloning of  
WT\*

<400> 7

gactgtacgc atatgaatgg accaataata atgac

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<210> 8

<211> 35

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:5'-Primer for subcloning of  
KT3-11 and HTK

<400> 8

gactgtacgc atatgaaagg accaataata atgac

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<210> 9

<211> 35

<212> DNA

<213> Artificial Sequence

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<223> Description of Artificial Sequence:3'-Primer for subcloning

<400> 9

gactgtacgc tcgagcgtaa ccaacatgtat taaca

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<210> 10

<211> 759

<212> DNA

<213> *Staphylococcus aureus*

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<221> CDS

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<400> 10

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Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

1

5

10

15

cat gaa att aag gaa cga ata ttg gat aaa tat ggg gat gat gtt aag

96

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys

20

25

30

gct att ggt gtt tat ggc tct ctt ggt cgt cag act gat ggg ccc tat

144

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr

35

40

45

tcg gat att gag atg atg tgt gtc atg tca aca gag gaa gca gag ttc

192

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe

50

55

60

agc cat gaa tgg aca acc ggt gag tgg aag gtg gaa gtg aat ttt gat	240		
Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp			
65	70	75	80
agc gaa gag att cta cta gat tat gca tct cag gtg gaa tca gat tgg	288		
Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp			
85	90	95	
ccg ctt aca cat ggt caa ttt ttc tct att ttg ccg att tat gat tca	336		
Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser			
100	105	110	
ggt gga tac tta gag aaa gtg tat caa act gct aaa tcg gta gaa gcc	384		
Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala			
115	120	125	
caa acg ttc cac gat gcg att tgt gcc ctt atc gta gaa gag ctg ttt	432		
Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe			
130	135	140	
gaa tat gca ggc aaa tgg cgt aat att cgt gtg caa gga ccg aca aca	480		
Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr			
145	150	155	160
ttt cta cca tcc ttg act gta cag gta gca atg gca ggt gcc atg ttg	528		
Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu			
165	170	175	

att ggt ctg cat cat cgc atc tgt tat acg acg agc gct tcg gtc tta 576

Ile Gly Leu His His Arg Ile Cys Tyr Thr Ser Ala Ser Val Leu

180

185

190

act gaa gca gtt aag caa tca gat ctt cct tca ggt tat gac cat ctg 624

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu

195

200

205

tgc cag ttc gta atg tct ggt caa ctt tcc gac tct gag aaa ctt ctg 672

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu

210

215

220

gaa tcg cta gag aat ttc tgg aat ggg att cag gag tgg aca gaa cga 720

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg

225

230

235

240

cac gga tat ata gtg gat gtg tca aaa cgc ata cca ttt 759

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe

245

250

<210> 11

<211> 253

<212> PRT

<213> Staphylococcus aureus

<400> 11

Met Asn Gly Pro Ile Ile Met Thr Arg Glu Glu Arg Met Lys Ile Val

1

5

10

15

His Glu Ile Lys Glu Arg Ile Leu Asp Lys Tyr Gly Asp Asp Val Lys

20

25

30

Ala Ile Gly Val Tyr Gly Ser Leu Gly Arg Gln Thr Asp Gly Pro Tyr

35

40

45

Ser Asp Ile Glu Met Met Cys Val Met Ser Thr Glu Glu Ala Glu Phe

50

55

60

Ser His Glu Trp Thr Thr Gly Glu Trp Lys Val Glu Val Asn Phe Asp

65

70

75

80

Ser Glu Glu Ile Leu Leu Asp Tyr Ala Ser Gln Val Glu Ser Asp Trp

85

90

95

Pro Leu Thr His Gly Gln Phe Phe Ser Ile Leu Pro Ile Tyr Asp Ser

100

105

110

Gly Gly Tyr Leu Glu Lys Val Tyr Gln Thr Ala Lys Ser Val Glu Ala

115

120

125

Gln Thr Phe His Asp Ala Ile Cys Ala Leu Ile Val Glu Glu Leu Phe

130

135

140

Glu Tyr Ala Gly Lys Trp Arg Asn Ile Arg Val Gln Gly Pro Thr Thr

145

150

155

160

Phe Leu Pro Ser Leu Thr Val Gln Val Ala Met Ala Gly Ala Met Leu

165

170

175

Ile Gly Leu His His Arg Ile Cys Tyr Thr Thr Ser Ala Ser Val Leu

180

185

190

Thr Glu Ala Val Lys Gln Ser Asp Leu Pro Ser Gly Tyr Asp His Leu

195

200

205

Cys Gln Phe Val Met Ser Gly Gln Leu Ser Asp Ser Glu Lys Leu Leu

210

215

220

Glu Ser Leu Glu Asn Phe Trp Asn Gly Ile Gln Glu Trp Thr Glu Arg

225

230

235

240

His Gly Tyr Ile Val Asp Val Ser Lys Arg Ile Pro Phe

245

250

*a*  
*cont*